

## Application of RNA probes to respiratory syncytial virus (RSV) vaccines: a novel nanotechnology approach for co-delivery of antigen and adjuvant

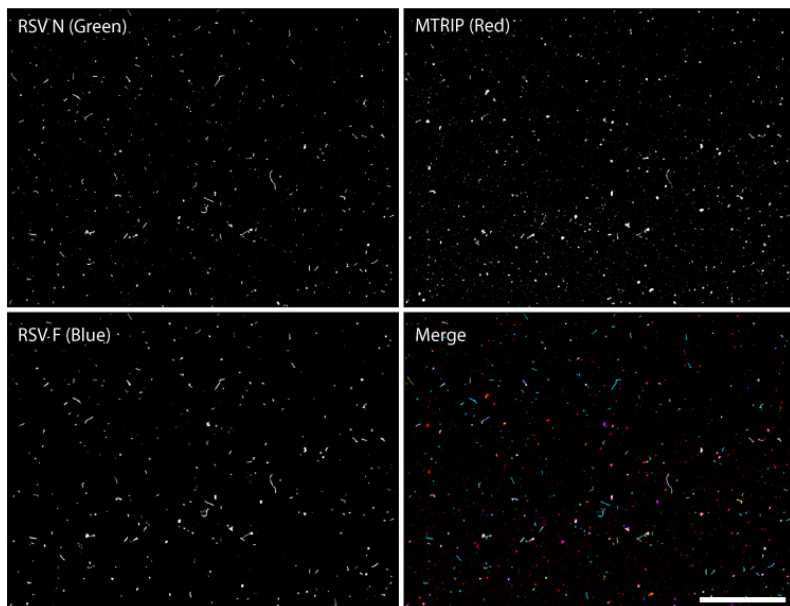
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**Specific Aims.** Respiratory syncytial virus (RSV) is the most important pathogen for lower respiratory tract illness (LRI) in infants. RSV causes LRI in 20-30% of infants and hospitalizes 1-3% of infants, resulting in ~120K hospitalizations/year in the USA (Glezen, Taber et al. 1986; Shay, Holman et al. 1999; Hall 2001). RSV causes 9 times more deaths in infants than influenza viruses. (Thompson, Shay et al. 2003) Therapies are not widely available, and there are no approved vaccines. Inactivated and subunit vaccines can cause immunopathology. In the 1960s, a formalin-inactivated RSV vaccine (FI-RSV) tragically resulted in vaccine enhanced RSV disease (ERD) upon natural RSV infection (Kapikian, Mitchell et al. 1969; Kim, Canchola et al. 1969). Live attenuated RSV vaccines do not cause ERD but suffer from genetic instability and surprisingly poor immunogenicity typical of wild-type (wt) RSV. A new mantra in the field is that a RSV vaccine will have to be more immunogenic than wt RSV. New approaches are urgently needed for RSV vaccines.

It was recently shown that immunization with inactivated RSV concurrent with toll-like receptor (e.g. TLR3) stimulation leads to enhanced immune responses. (Delgado, Coviello et al. 2009) TLR3 is a protein that recognizes double-stranded (ds) RNA and replicating RNA viruses. The minimum length of dsRNA needed to create to stimulate TLR3 is at least 21 nucleotides (Kleinman, Yamada et al. 2008). Here, we will create a RSV vaccine by incorporating RNA probes into RSV virions, rendering portions of the normally single-stranded viral RNA genome double-stranded. Our hypothesis is that modified ds RNA RSV (mdsRSV) will elicit a greater TLR3 response and be more immunogenic than wt RSV. Thus, we will modify RSV by incorporating RNA probes into the virus itself to make the virus a better TLR3 agonist, a novel approach for targeted co-delivery of antigen and adjuvant.

Aim 1. Generate modified RSV with regions of ds RNA in viral genome, mdsRSV.

**Results to date:** To date we have been able to reliably generate three different vaccine candidates: 1) virus 1 (mdsRSV-23) was loaded with genomic RNA containing multiple 23 nt double-stranded sections, and 2) virus 2

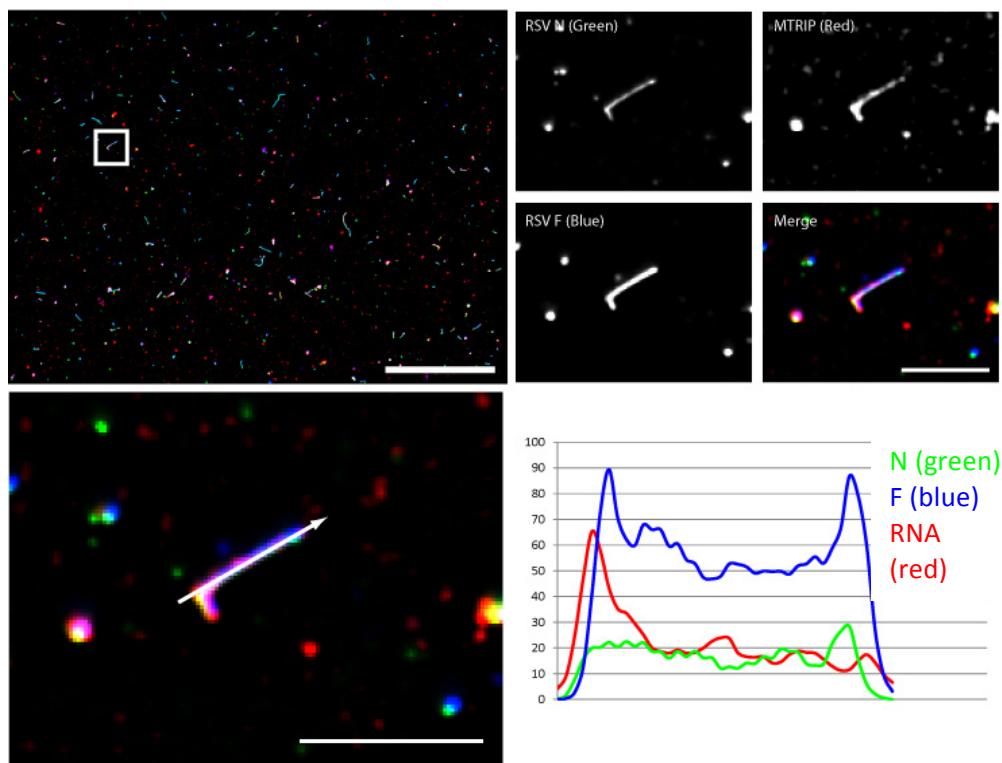


**Figure 1.** RSV virion centrifuged onto poly-L Lysine coated coverslips, isolated from HEp-2 cells 48 hours post-infection and 30 minutes after RNA probes were delivered. After centrifugation, the coverslips are washed, fixed in 1% paraformaldehyde, and permeabilized with Triton-X. They are then stained for the F and N proteins. Here, the N is represented as green, the F is blue, and the RNA probe (MTRIP) red. Virions were imaged via deconvolution microscopy using a 63x, NA=1.4 objective.

(mdsRSV-46) was loaded with genomic RNA containing one 46 nt double-stranded section per genomic RNA, and 3) virus 3 (mdsRSV-MTRIP) was loaded with MTRIP probes that bind to a repeated (3x) 17 nt section of the viral genome.

Virus 1 and 2 were then tested *in vitro* using the HEK blue-TLR3 cell line from InVivoGen. These results are discussed later in this report.

Another important discovery was that both our MTRIP probes (Figure 1 and 2) and nanogold-labeled MTRIPs could be efficiently incorporated into the RSV virion, without decreasing the titer of the virus. Incorporation was confirmed via fluorescence microscopy, and TEM is currently being performed. This opens a number of possibilities that we did not anticipate, such as the incorporation of other macromolecules such as poly AU strands (which we have acquired and will be testing shortly), and imidazole, a TLR7 agonist, which can be directly conjugated to our probes. These two agents in combination may be much stronger stimulators of TLR 3 and 7 producing higher affinity antibodies against RSV.



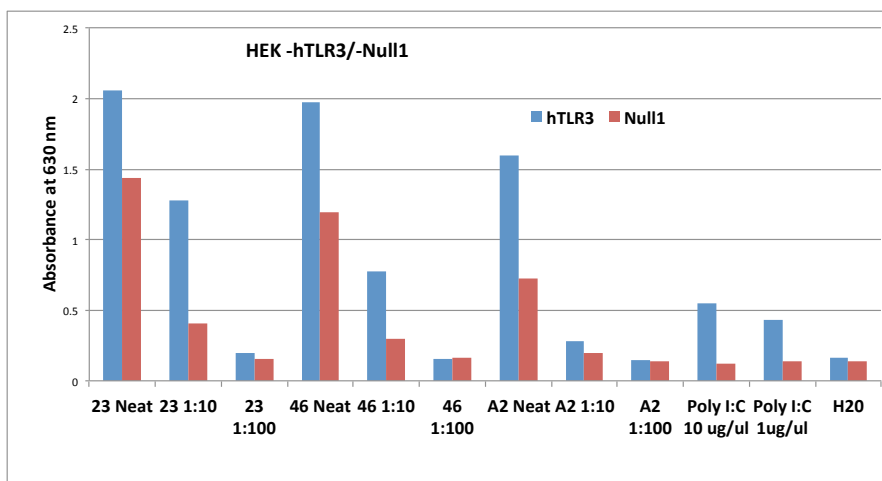
**Figure 2.** Zoomed in view of a partially branched, RSV viral filament, showing the distribution of the N, F and RNA along the filament.

**Aim 2.** Determine the ability of dsRSV to stimulate TLR3 signaling in vitro, and determine the immunogenicity and protective efficacy of dsRSV vaccine *in vivo*.

**Aim 2A.** The Moore lab (Emory) will quantify TLR3 activation by RSV and mdsRSV (23 and 46 nt dsRNA) in epithelial cells and dendritic cells in vitro. Poly (I:C), a dsRNA analog and known agonist of TLR3, will serve as a positive control. **Aim 2B.** The Moore lab (Emory) will quantify immune responses and protective efficacy induced by dsRSV, wt RSV, FI-dsRSV, and FI-RSV. Immunogenicity and protective efficacy of the dsRSV vaccine will be tested in a BALB/c mouse model of RSV pathogenesis.

#### Results to date:

To date, the mdsRSV-23 and 46 have been tested in a stable 293T cell line stably transfected with human TLR3



**Figure 3.** Results of testing of vaccine viruses, 23 and 46, wild-type virus, A2, and Poly I:C

and a secreted reporter of NF- $\kappa$ B activation. As seen in Figure 3, mdsRSV-23 and 46 elicited a greater TLR3 response than wild type-A2 and PolyI:C. They also stimulated the null cells but this could be a result of TLR7 or RIG-I-like receptor signaling induced by the additional nucleic acids delivered with the virion. This experiment will be repeated shortly and then moved to an animal model. In addition, we will compare these results with those of RSV with Poly AU incorporated within the virion.

**Future steps:** In the next few months the vaccine viruses produced will be retested in vitro and if the results are repeatable, they will then be tested in mice as per Aim 2B. We will also test the use of Poly AU and imidazole as well. If the results look promising, a publication on the basic approach will be assembled and an R21 grant written for NIH.

## References

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